PREVALENCE AND DISTRIBUTION OF BOVINE TUBERCULOSIS AMONG SLAUGHTERED CATTLE IN CROSS RIVER STATE, NIGERIA

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ABSTRACT

Tuberculosis (TB) in slaughtered cattle (SC) is of immense zoonotic and economic importance worldwide. This study determined the prevalence and distribution of TB among SC in three agricultural zones of Cross River State, Nigeria, using post mortem lesions, the Ziehl-Neelsen staining technique (ZNST) and retrospective abattoir records. Out of the 1,852 SC surveyed from April, 2013 – March 2014, 4.10 % had TB-like lesions, out of which 86.8 % were positive when subjected to ZNST. Similarly, a 9 year retrospective survey (2001 – 2010) using abattoir records revealed 1.00 % prevalence of bovine tuberculosis (BTB) in the State. The periodic prevalence of the infection were: (2001– 2002) 1.0 %, (2003) 0.9 %, (2004 – 2005) 0.8 %, (2006 – 2007) 1.2 % and (2008 – 2010) 1.1 %. The survey revealed higher BTB prevalence in dry (6.09 %) than wet (2.15 %) season, in cows (6.20 %) than bulls (2.60 %), in older cattle >3 years (7.86 %) than younger ones ≤3 years (1.16 %), in White Fulani (4.52 %) than Red Bororo (3.95 %) and mixed breed (3.07 %). Similarly, higher rates of 1.26 % in dry than 0.77 % in wet season, 1.61 % in cows than 0.63 % in bulls, 1.27 % in cattle ≤ 3 years than 0.65 % in those > 3 years were recorded in the retrospective study. BTB was prevalent in SC in the State and this portends significant health risk and economic losses to the populace and necessitates a multi-sectorial One Health approach to its control.

Keywords: Bovine tuberculosis, Cross River State, Prevalence, Slaughtered cattle, Zoonoses

INTRODUCTION

Tuberculosis (TB) in cattle is of huge public health and economic consequences especially in developing countries (Adesokan *et al*., 2018). In Nigeria, poor diagnostic facilities and inadequate trained manpower have contributed immensely to the endemicity and rising trend of most infectious diseases, especially zoonotic TB (Olea-Popelka *et al*., 2017; Kruk *et al*., 2018). Bovine tuberculosis (BTB) is a chronic debilitating disease of cattle caused mainly by *Mycobacterium bovis;* a fungus-rod like organism (Ahmad *et al*., 2017). However, *Mycobacterium tuberculosis* which causes TB in humans as well as other members of the *M. tuberculosis* complex have also been isolated from cattle implicated for BTB (Cadmus *et al.,* 2010a; Jenkins *et al.,* 2011; Ibrahim *et al.*, 2012; Krajewska *et al*., 2012; Gelalcha *et al.*, 2019). Depending on the immune status of the infected animal, obvious clinical signs may be absent. However, clinical manifestation may include weakness, labored breathing, anorexia, un-thriftiness and persistent cough. Others are lymphadenopathy which causes obstruction of air passages, alimentary canal, or blood vessels particularly at the head and neck regions hence worsen the animal's condition (Ramos *et al.*, 2015). Multiple foci of caseous necrosis in the lung are pathognomonic (Ramos *et al.*, 2015). However, in generalized TB, extra-pulmonary lesions, like pin-point tubercles may be present on all the organs of the gastrointestinal tract (Cantres-Fonseca *et al.*, 2018).

Tuberculosis in cattle has been associated with huge economic losses due to reduced productivity, cost of screening and culling of infected animals, poor meat yield and condemnation of affected carcasses at slaughter (Ibironke and Fasina, 2010; Ejeh *et al.*, 2014). An annual losses of 13 – 24 million Naira due to condemnations of TB affected meat have been reported in Nigeria (Cadmus and Adesokan, 2009; Raufu and Ameh, 2010). These may therefore worsen the already precarious animal protein need in most developing countries and deny people of their major means of livelihood (Majekodunmi *et al.*, 2017; Gwaka and Dubihlela, 2020). It further possess challenges on trade especially in Nigeria, where the livestock industry generates about 94 % of the animal protein, contributing between 4 – 5 % to the national gross domestic product (FGN, 2009). Furthermore, the country has been rated 4th with TB-burden in the world and the first in Africa with 2.8 % of human TB cases attributed to *M. bovis* (Abubakar *et al.*, 2011; Miller *et al.*, 2013; Oyefabi *et al.*, 2017). The constant uncontrolled trans-border influx of herders and cattle from the neighboring states and countries without inspection and access to veterinary services may have contributed to the endemicity of the disease in Nigeria (Nwanta *et al.*, 2011).

Zoonotic TB can be transmitted via inhalation of infected aerosol droplets, close-contact and sharing of environment with infected animal as well as by the consumption of contaminated meat and milk (El-Sayed *et al.*, 2016; Yahyaoui-Azmi *et al.*, 2017). The onset of signs and symptoms of *M. bovis* infection in humans is usually delayed except in immune-comprised individuals (WHO, 2018). Similar clinical forms as those caused by *M. tuberculosis* have been reported (Grange, 2001; FMOH, 2016; Torres-Gonzalez *et al.*, 2016; Adane *et al.*, 2020). The pulmonary form of the disease condition occurs less frequently but is usually occupationally related (Singh *et al.*, 2013).

In 2016, WHO estimated that 147,000 new human cases of zoonotic TB occurred globally and around 12,500 people died of the disease the same year (Teppawar *et al.*, 2018). The true burden of zoonotic TB is likely to be underestimated due to lack of routine surveillance data in most countries. Therefore, the number of people affected by zoonotic TB annually, and those suffering health challenges caused by *M. bovis*, might be higher than currently estimated especially in the Sub-Saharan Africa and the Southeast Asian region, where laboratory capacities are limited (Cosivi *et al.*, 1998). This is in contrast to the developed countries where there are high food safety standards and BTB eradication programs are implemented (de la Rua-Domenech, 2006).

In zoonotic TB endemic countries, the natural resistance of *M. bovis* to pyrazinamide, one of the four essential medications used in the standard first-line anti-TB treatment regimen is a challenge (Bobadilla-del Valle *et al.*, 2015). In addition, the complex relationship between humans, livestock, wildlife and the environment in the epidemiology of BTB has made the control of the neglected zoonosis multifarious (Miller *et al.*, 2013; Mohamed, 2020). In this regard, the determination of the prevalence and distribution of TB among slaughtered cattle (SC) in Cross River State, Nigeria as conducted in this study will add to the limited data to be use in the effective control of the disease in Nigeria.

MATERIALS AND METHODS

Study Area: Cross River State is located between latitude $4^0 – 7^0$ North and longitude $14^0 – 15^0$ East. The State is bordered in the North by Benue State, in the South by Akwa Ibom State, Ebonyi State in the West and by Cameroon in the East. The State comprises of 18 Local Government Areas and three agricultural zones namely Ogoja, Ikom and Calabar. The State has tropical climate characterized by two distinct seasons; dry season lasts for a minimum of five months (October – March), while the wet season spans from April – September. Cross River State has a population of about 3 million people of diverse ethnic groups, majority of these are Efik and Ejagham (NPC, 2006). The increase influx of people and cattle into the State from the Republic of Cameroon has led to increased demands and sales of beef and other social activities in the State (Figure 1).

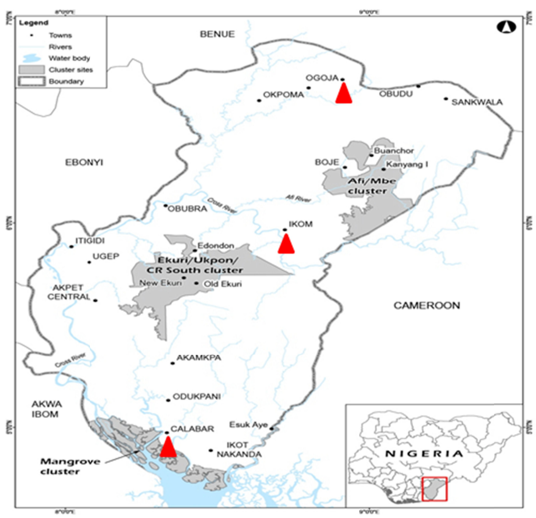

Figure 1: Map of Cross River State showing the eighteen Local Government Areas within the three agricultural zones of the state. The studied agricultural zones are indicated by a red triangle. Source: Modified from Ogbaji *et al.* (2017)

Study Design: A cross sectional study involving retrospective evaluation of abattoir records and survey for TB-like lesions in SC across the three agricultural zones of Cross River State were conducted to determine the prevalence and distribution of BTB. The survey for TB-like lesions suggestive of BTB lasted for a period of 12 months (April 2013 – March 2014), while a retrospective assessment on the infection among slaughter cattle was equally investigated using abattoir records over a period of 9 years (2001 – 2010).

Sample Size: The sample size was calculated using the formula: $n = Z^2P(1 - P)/d^2$ (Thrusfield, 2007), where N = sample size, z = risk of type 1 error (= 1.96 at 95 % confidence level) p = previous prevalence of BTB = 2.40 % (Bikom and Oboegbulem, 2007), d = precision (allowable error) = 5 % = 0.05. A minimum sample size of 438 was calculated. However, 1,852 SC were examined during meat inspection, while the BTB infection status of 117165 SC between 2001 and 2010 was determined from abattoir records.

Survey Study: Slaughterhouses in each agricultural zone were identified and one per zone was selected purposively based on high slaughter capacity. Weekly visits to the selected slaughterhouses revealed the average slaughter capacity of (40, 20 and 20) cattle respectively for Calabar, Ikom and Ogoja, given a total slaughter capacity of 80 x 52 weeks or 4, 160 cattle between April 2013 and March 2014. One in every four of the SC, selected by systematic random sampling, was examined at post-mortem for lesions of TB such as presence of nodules, tubercles in the lung and enlargement of other internal organs. In each selected cattle, the breed, age and sex were determined and recorded as described by Nwankwo *et al.* (2019). The BTB suggestive lesions were collected and the samples transported in cold and sterile conditions to the Veterinary Public Health Laboratory, University of Nigeria for identification of the presence of acid-fast bacilli (AFB) using Ziehl-Neelsen staining technique (ZNST) (Ahmad *et al.*, 2017).

Ziehl-Neelsen Acid Fast Staining Technique: Five smears of the homogenates (sediment) of each specimen were made and stained with by Ziehl-Neelsen stain as described by Ahmad *et al.* (2017). The stained slides were viewed under x100 objective of a light microscope to determine the presence and morphology of acid-fast bacilli. The presence of typical pinkish rod-shaped organisms under a blue-black background (when methylene blue was used as a counter stain) indicated acid fast positivity and hence confirmatory of BTB (Kazwala *et al.,* 1998).

Retrospective Study: Records of slaughter and meat inspection at abattoirs (Calabar, Ikom and Ogoja) over a period of 9 years (2001–2010) were abstracted from the Veterinary Division at the Headquarters of Ministry of Agriculture and Natural Resources, Calabar including the total SC, month of slaughter, number of TB-suspected cases, sex, age and breed of the cattle.

Data Analysis: Data obtained were analyzed using Open Source Epidemiologic Statistics for Public Health (OpenEpi), Version 3.0 (Centre for Disease Control and Prevention, CDC). Association between BTB infection and the 3 agricultural zones of the state as well as season, sex, age and breeds of cattle were determined using Chi-square test and significance was accepted at $p<0.05$.

RESULTS

Out of 1,852 SC surveyed in abattoirs across the three agricultural zones of Cross River State, a total prevalence of (4.10 %) (76/1852) had BTB lesion of which 66 (86.84 %) had acid-fast bacilli in them. Furthermore, the prevalence and distribution across the agricultural zones revealed 30(3.25 %), 24(4.22 %) and 22(6.11 %) out of 923, 569 and 360 SC in Calabar, Ikom and Ogoja agricultural zones respectively. However, the strength of association between the infections across the zones of the state was not significant ($p>0.05$) (Table 1).

On the other hand, out of the 117165 SC between the periods of 9 years (2001 – 2010), 1.00 % (1155/117165) prevalence of TB was indicated using abattoir records. A monthly prevalence range of 0.0 to 9.25 % which peaked in the month of March was recorded, while a decrease in prevalence of TB from 1.0 % in 2001 – 2002 to 0.80 % in 2005, which peaked at 1.20 % in 2006 – 2007 and drop to 1.1 % in 2008 – 2010 was recorded (Figures 2 and 3).

The survey study revealed 20(2.15 %) and 56(6.07 %) positive for TB out of 932 and 920 SC examined during the rainy and dry season respectively, while the retrospective study revealed 431(0.77 %) and 724(1.26 %) out of 55,906 and 57,555 SC respectively during the rainy and dry seasons in the same order. The association between TB prevalence and the seasons were statistically significant ($p<0.05$) (Table 2).

Furthermore, out of 739 and 1113 male and female SC surveyed, 46(6.22 %) and 30(2.70 %) were TB positive respectively, while the overall sex distribution of 723(1.61 %) and 432(0.63 %) out of 44,794 and 68,667 were for cows and bulls in the same order retrospectively. The association between TB in SC and the sex was statistically significant ($p<0.05$) (Table 3).

Furthermore, out of 1038 and 814 cattle aged ($\leq$3 years) and (> 3 years) surveyed, 12(1.16 %) and 64(7.86 %) were TB positive respectively, while the age distribution showed 851(1.27 %) and 304(0.65 %) were positive for TB out of 66,994 cattle > 3 years and 46,467 cattle $\leq$ 3 years in that same order retrospectively. The association between TB and age of cattle was statistically significant ($p<0.05$) (Table 4).

The distribution of TB among the SC breeds surveyed revealed the highest prevalence of 4.52 % in White Fulani breed, followed by Sokoto Gudali (4.19 %), Red Bororo (3.95 %) and mixed breed (3.07 %), while Muturu and Bokolo had zero prevalence. The abattoir records revealed TB in 6(0.66 %) out of 911 Red Bororo, 10(0.44 %) out of 2,272 Bokolo, 180(0.93 %) out of 19319 mixed breed, 94(0.83 %) out of 11364 Sokoto Gudali, 864(1.09 %) out of 79368 White Fulani and 1(0.44 %) out of 227 Muturu breeds of cattle retrospectively. The association between TB and breeds of SC were statistically significant ($p<0.05$) (Table 5).

Table 1: Prevalence and distribution of bovine tuberculosis among slaughtered cattle surveyed in Cross River State, Nigeria

Agricultural zones	Number examined	Number of tuberculosis (Negative cases)	Number of tuberculosis (Positive cases)(%)	P-value
Calabar	923	893	30(3.25)	0.07
Ikom	569	545	24(4.22)	
Ogoja	360	338	22(6.11)	
Total	1852	1776	76(4.10)	

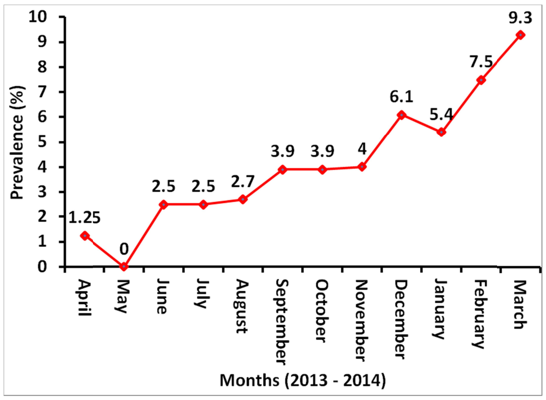

Figure 2: Monthly prevalence of bovine tuberculosis lesions among slaughtered cattle in Cross River State, Nigeria (2013 – 2014)

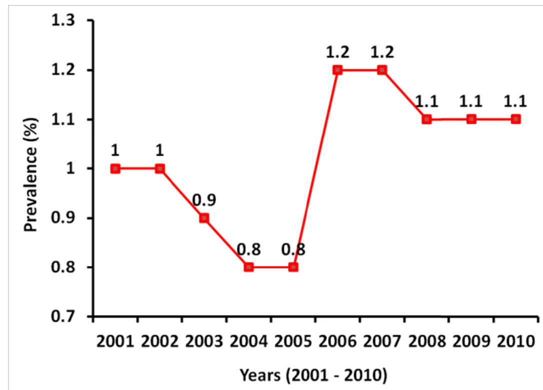

Figure 3: Annual prevalence of bovine tuberculosis among slaughtered cattle in Cross River State, Nigeria (2001– 2010)

Table 2: Seasonal prevalence of bovine tuberculosis among slaughtered cattle in Cross River State, Nigeria

Methods of study	Seasons	Number of tuberculosis negative cases	Number of tuberculosis positive cases (%)	OR	95 % CI	P-value
Survey	Wet (April – September)	912	20(2.15)	1		
	Dry (October – March)	864	56(6.09)	2.96	1.76 – 4.97	0.00
Total			76(4.10)			
Abattoir record	Wet (April – September)	55475	431(0.77)	1		
	Dry (October – March)	56831	724(1.26)	1.64	1.46 – 1.85	0.00
Total		114082	1155(1.02)			

Table 3: Sex distribution of bovine tuberculosis among slaughtered cattle in Cross River State, Nigeria

Methods of study	Sex	Number of cattle examined	Number of tuberculosis negative cases	Number of tuberculosis positive cases (%)	OR	95 % CI	P-value
Survey	Male	739	693	46(6.22)	1		
	Female	1113	1083	30(2.70)	0.42	0.26 – 0.67	0.00
Total		1852		76(4.10)			
Abattoir record	Male	68667	68235	432(0.63)	1		
	Female	44794	44071	723(1.61)	2.59	2.29 – 2.92	<0.00
Total		117165		1155(1.00)			

Table 4: Age distribution of bovine tuberculosis among slaughtered cattle in Cross River State, Nigeria

Methods of study	Age (years)	Number of cattle examined	Number of tuberculosis negative cases	Number of tuberculosis positive cases (%)	OR	95 % CI	P-value
Survey	≤3	1038	1026	12(1.16)	1		
	> 3	814	750	64(7.86)	7.296	3.91 - 13.61	<0.00
Total		1852		76(4.10)			
Abattoir record	≤ 3	66994	66143	851(1.27)	1		
	> 3	46467	46163	304(0.65)	0.5118	0.45 – 0.58	<0.00
Total		117165		1155(1.00)			

Table 5: Breed distribution of bovine tuberculosis among slaughtered cattle in Cross River State, Nigeria

Breeds	Methods of study A/B	Number of cattle examined	Number of tuberculosis negative cases	Number of tuberculosis positive cases (%)	P-value
White Fulani	A	1282	1224	58(4.52)	<0.00
	B	79368	78504	864(1.09)	
Mixed	A	261	251	8(3.07)	<0.00
	B	19319	19139	180(0.93)	
Red Bororo	A	253	243	10(3.95)	<0.00
	B	911	905	6(0.66)	
Bokolo	A	76	76	0(0.00)	<0.00
	B	2272	2262	10(0.44)	
Muturu	A	20	20	0(0.00)	<0.00
	B	227	226	1(0.44)	
Sokoto Gudali	A	1812	1736	76(4.19)	<0.00
	B	11364	11270	94(0.83)	
Total		117165	115856	1155(1.00)	

Note: *A = Survey, B = Abattoir record*

DISCUSSION

The study has revealed the prevalence of TB among SC at 4.10 % in a year survey as well as 1.0 % in 9 year retrospective record. This is rather of serious public health concern as Nigeria has been ranked 6th out of the eight countries with two third of the total number of new TB cases worldwide in 2019 (WHO, 2020). Moreover, the country is yet to have a national control policy in place (WHO, 2020). Even though the prevalence of 1.0 % in the retrospective study (2001 – 2010) was lower than 2.5 % recorded (1991 – 2000) in the same study area (Bikom and Oboegbulem, 2007), it was still higher than 0.9 % projected for the developing countries like Nigeria in 2005 (Ofukwu, 2006).

Furthermore, the need for active surveillance has been justified by the 4.10 % TB prevalence in the survey since factors like poor and unreliable records have been often associated with retrospective studies (Bikom and Oboegbulem, 2007; Aliyu *et al.,* 2009; Awah-Ndukum *et al*., 2010). The reported prevalence in this study may have been as a result of unrestricted inflow of herders with their cattle from the neighboring states as well as across the international boundaries of Cameroun where TB prevalence of 0.67 – 4.28 % in SC have been reported (Awah-Ndukum *et al*., 2005; 2010). This can be supported by the gradual increase in the prevalence of TB among the SC across the agricultural zone based on their distances from the international border of Cameroun. The least (3.25 %) was at Calabar agricultural zone which borders directly with Cameroun, followed by Ikom agricultural zone (4.22 %), while the uppermost (6.11 %) was at Ogoja agricultural zone. From the study, less number of chronic diseased cattle were slaughtered in Calabar agricultural zone which has double slaughter capacity than the other two agricultural zones. Latently infected cattle can build up granulomatous tissues as they move further into the state or stay longer before slaughter. Therefore, there is need for strict animal movement control as well as systematic tuberculin skin testing both at point of animal entry and at the herd level in the state in addition to active surveillance at abattoirs (Koleci and Koni, 2018). The high prevalence level is of huge economic consequences and public health risk especially for farmers in the state who may have been infected due to their occupation thereby increasing the chances of the infection spread from infected to uninfected herds and people in the community (Awah-Ndukum *et al*., 2012; Singh *et al*., 2013). Meanwhile, a study in the study area had reported TB prevalence of 24.0 % among out patients attending hospital for medication (Kooffreh *et al*., 2016).

The 1.0 % prevalence in this study, agreed with 1.4 % reported in Enugu State, Nigeria (Nwanta *et al*., 2011), but lower than 0.54 – 0.57 % and 0.34 % in Ogbomoso Area of Oyo State and Adamawa State respectively (Ameen *et al*., 2008; Aliyu *et al.,* 2009). However, much higher BTB prevalence of 11.2 % has been reported in Plateau State using abattoir record (Okeke *et al*., 2016). The gradual decrease from 1.0 % in 2001/2002 to 0.8 % in 2005, which peaked at 1.2 % in 2006/2007 and decreased to 1.1 % from 2008 – 2010, disagreed with the average yearly prevalence of 9.1 % between 2007 – 2012 (Okeke *et al*., 2016). Furthermore, the range of 0 – 9.3 % monthly prevalence that peaked in the month of March was equally in disagreement with a reported peak in July and August by Okeke *et al*. (2016). The fluctuations in BTB prevalence both in months and years as reported in this study may be associated with the uncontrolled tracking of TB in live cattle in Nigeria and particularly in the study area where unhealthy and unproductive cattle are usually the first to be removed from herds and slaughtered for meat production (Awah-Ndukum *et al*., 2012).

The 4.10 % prevalence in the survey study agreed with that of Damina *et al.* (2011) who reported 4.4 % among SC in Plateau State, Nigeria and 4.4 % reported by Okoro *et al.* (2014) in Enugu State, Nigeria. However, the prevalence was lower than 6.1 % reported among SC in Zamfara State, Nigeria (Ahmad *et al*., 2017) as well as higher values of 17.3 % and 14.0 % in Abuja and Kaduna respectively (Abubakar *et al*., 2005). The result of the survey was also similar to the study done by Cadmus *et al.* (2010b) in Southwest Nigeria and Aliyu *et al.* (2009) in the Northern State of Nigeria who reported BTB prevalence of 4.30 and 4.10 % respectively using abattoir records. The differences in the prevalence have been associated with the disease status of the SC, environmental factors and the methodology employed during the study (Awah-Ndukum *et al*., 2012).

A comparison of the study with other studies including a previous study in the same study area showed that BTB in SC had seasonal variations (Bikom and Oboegbulem, 2007; Awah-Ndukum *et al*., 2012; Okeke *et al*., 2016). However, the study disagreed with Nwanta *et al*. (2011) that reported no seasonal difference in the prevalence of bovine and human TB in abattoirs and hospitals in Enugu State, Nigeria. The seasonal prevalence obtained in the study was lower than 4.7 and 3.7 % in the dry and wet seasons respectively as reported by Ofukwu (2006) in Benue State, Nigeria. The higher rate in the dry season may be associated with the cold dry and windy harmmattan in the Northern part of the state which could lead to inhalation of infected dust that can elicit the disease in the respiratory system, indiscriminate grazing and congregation of large herd of cattle in the same drinking points during the dry seasons (Cook *et al.,* 1996; Abubakar, 2007). Furthermore, there was strong association between sex and prevalence of BTB among SC in the agricultural zones of the state agreed with similar studies in Enugu State, Nigeria (Nwanta *et al.,* 2001), Sokoto State, Nigeria (Garba, 2002), Benue

State, Nigeria (Ofukwu, 2006), Cameroon (Awah-Ndukum *et al*., 2012), Adamawa State, Nigeria (Ejeh *et al*., 2013) and Zamfara State, Nigeria (Ahmad *et al*., 2017). However, absence of strong association between sex and BTB lesions has been reported in Ogbomoso area of Oyo State (Ameen *et al*., 2008). The higher prevalence in females than the males in this study may be linked to the reproductive value of females as they usually remain in the herd longer than the males thereby becoming more exposed to the infection. However, higher prevalence in male than female has been attributed to higher slaughter rate of bulls than the cows in Maiduguri, Borno State (Abubakar *et al*., 2011).

The high prevalence of BTB among the older SC may be due to its insidious nature and the extraordinary ability of *Mycobacteria* to survive for a longer period of time and be revealed in advanced stages. Garba (2002) had reported that older cattle are more at risk of BTB than the young ones. The higher prevalence of BTB in older SC of age > 3 years agreed with the studies of Awah-Ndukum *et al*. (2012), Okoro *et al.* (2014) and Ahmad *et al*. (2017) who reported higher prevalence among adult/older cattle than in younger ones.

The highest prevalence among breeds that occurred in the White Fulani breed (4.52 %) agreed with the studies of Garba (2002) in Sokoto State, Okoro *et al.* (2014) in Enugu State and Ahmad *et al*. (2017) in Zamfara State, Nigeria that revealed the highest prevalence in White Fulani breed. However, it disagreed with the study of Ofukwu (2006) that reported the highest prevalence in the Sokoto Gudali breed. The least prevalence range of 0.00 – 0.44 % in Muturu breed in the study signifies high resistant by the breed, and agreed with the study of Vordermeier *et al*. (2012) who reported more resistant to BTB by the native Zebu cattle than the exotic breed in Ethiopia.

Conclusion: BTB was prevalent at 4.10 and 1.00 % using survey and abattoir record respectively among SC in the three agricultural zones of Cross River State, Nigeria. The study has added to the base line data and further revealed the need for active surveillance of BTB towards effective control in the state and the country at large. This is needful considering the underestimated zoonotic implications of BTB.

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